MOBILIZATION OF DEFENSIVE CELLS IN INFLAMMATORY TISSUE

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The present poverty of the classical techniques of morbid anatomy is nowhere better illustrated than in the study of inflammation. The essential characteristics of the emigration of polymorphonuclear leucocytes from vessels were described by the middle of the 19th century (1–3); and the association of monocytes with the lesions of leprosy, typhoid, and tuberculosis was described at about the same time (4–6). Chemotaxis of polymorphonuclear leucocytes was described by Leber in 1888 (7). In 1892 Metchnikoff delivered the classical lectures in which the phagocytic role of polymorphonuclear leucocytes and monocytes was elucidated (8). Collections of eosinophilic leucocytes in the tissues in certain pathological conditions had been described by the 1890's (9–12), and by 1901 the general doctrine had already been established that lymphocytes accumulated in the tissues wherever there was prolonged cellular disintegration (13). Descriptions of lymphocytes in various forms of chronic inflammation had, of course, been given earlier (14–16).

The following features of inflammation were therefore well known by the beginning of the century:

a. Polymorphonuclear leucocytes adhered to the walls of inflamed blood vessels and emigrated through these walls into the tissues.

b. Polymorphonuclear leucocytes were attracted chemotactically toward many bacteria and were able to phagocytize and digest some of them.

c. Certain bacterial lesions were characterized by the accumulation of large numbers of mononuclear cells (the macrophages of Metchnikoff), and such cells were also seen during wound healing and in many forms of chronic inflammation.

d. The mononuclear cells showed phagocytosis of bacteria and also of tissue debris.

e. Eosinophile leucocytes collected in the tissues in a variety of parasitic infestations and also in certain other conditions such as bronchial asthma.

f. Cells indistinguishable from lymphocytes collected in and around chronic inflammatory lesions, certain tumors, and transplanted tissues.

The astonishing thing about this list is that it can, with very little addition, stand as a summary of contemporary knowledge of the role of defensive cells in inflammation. We have not yet determined:

a. Whether there are any specific chemical substances responsible for the mobilization of the polymorphonuclear leucocytes.

b. Whether monocytes emigrate from the vessels in the same way and at the same time as polymorphonuclear leucocytes or whether the emigration of monocytes is provoked by physical or chemical stimuli different from those which provoke the emigration of polymorphonuclear leucocytes.

c. The mechanism of eosinophile accumulation.

d. The mechanism of lymphocyte accumulation.

These four unresolved questions and some aspects of the information available about each of them will be discussed in this review. Certain possibilities for experiment will also be suggested. Of course, no one can really know whether an experimental approach is actually feasible until it is tried, but since our experimental record in the field of inflammation during the last 50 years is, on the whole, a poor one there may be some excuse for rashness.

CHEMICAL SUBSTANCES RESPONSIBLE FOR LEUCOCYTIC EMIGRATION

The notion that specific chemical substances released by injured cells are responsible for the observed phenomena of inflammation has a long history. It probably has its origin in the assumption that since the acute vascular responses to injury are similar whatever the nature of the injury, these responses must be mediated by a single chemical substance or group of substances —the essential common denominator of inflammation. The earliest definite enunciation of this theory is to be found in the work of Massart and Bordet in 1890 (17). These workers carried out experiments which purported to show that injured cells release substances which are chemotactic for leucocytes. There is a long overdue case

for making a close examination of the assumptions on which this theory and all subsequent variants of it are based.

It will be generally agreed that the first observable changes in inflammation are those affecting the nature of the blood flow. It will also be agreed that no adherence of leucocytes to vessel walls occurs until the passage of the leucocytes through the capillaries is slowed. The first problem, therefore, is to determine whether a specific substance released by the tissues is responsible for the initial vascular change.

Substances Responsible for Initial Vascular Response

All stimuli, whether chemical or physical, which can be grouped under the antique term "noxa" produce in varying degrees the initial vascular response of inflammation, and no one who has worked with the rabbit ear chamber will fail to appreciate how trivial a noxa will elicit this response. There seems to have been a general unwillingness to accept the possibility that the noxa acts directly on the vessels, and a large variety of substances has been proposed over the years as final mediator of the response. These substances include, *inter alia*, serum proteins, tissue proteins, enzymes, peptides, nucleotides, purines, and amines. The evidence in support of all these candidates rests on experiments of the same general type. The substance is introduced in some way into the tissues of some experimental animal and the increase in permeability of the vessels at the injection site is assessed by the extent to which they permit the passage of a protein-bound dye. If adherence or emigration of leucocytes is to be studied, histological sections of the injection site are made.

The possibility may be suggested that this type of experiment is, in principle, incapable of establishing the identity of the mediating substance if one exists. The fact that an injected substance produces the vascular response cannot decide whether the effect is produced directly by the injected substance or indirectly through another mediator. Nor can the two possibilities easily be resolved by an analysis of the severity or the rapidity of the response. A rapid response might mean a rapid release of the effector substance from the tissue cells and a severe response might mean that more of the substance or a more active substance is released. Although the array of compounds which have been canvassed as the true effector substance is imposing enough, it can be predicted that such compounds will continue to increase in number and variety until some more cogent assay of their effect of vessels is worked out than simply their injection into the tissues.

It is a counsel of perfection to suggest that these assays must be carried out on capillaries in the absence of other tissue cells in the same way as histamine may be assayed on an isolated strip of smooth muscle. One does not find capillaries in nature functioning in the absence of other tissue. But there are situations where leashes of capillaries are supported by very little other tissue and in some cases by no more than a layer or two of homogeneous cells, for example, in certain membranes. Such structures appear to be a more suitable type of assay material. They may not resolve the basic question of whether the noxa acts directly or through the release of substances by the cells, but they should permit experiments of a much more quantitative type, and thus perhaps permit a distinction to be made between a direct and an indirect response. With some reservations, the rabbit ear chamber might lend itself to this type of assay, if some technical development in the design of the chamber could permit the introduction of substances into it without trauma. In some circumstances the hamster cheek pouch might also be suitable. A word of warning is necessary about the type of vehicle in which the test substance is to be assayed. It seems not improbable that capillaries will prove to be sensitive to the pH, tonicity, temperature, protein concentration, electrolyte composition and other properties of the fluids bathing them, and great care may be necessary to control these factors. It should be remembered that for many types of mammalian cells "physiological saline" is a very corrosive fluid.

Substances Responsible for Leucocyte Emigration

The adherence of leucocytes to the vessel walls may involve a change in the surface character of the cells or the walls or both, but it does not necessarily involve such a change. Normal leucocytes might well adhere to normal capillary walls if they were brought into contact with them for long enough, as they are during the inflammatory response. But if a change in the surface character of at least one or other is necessary for adherence,

it seems more likely that the change would be in the capillary. This was Cohnheim's view, and it is perhaps supported by the fact that leucocytes isolated from shed blood are at once able to adhere to a wide variety of surfaces. The capacity of leucocytes to adhere to such surfaces can be reduced *in vitro* only by injuring the cells and no way of increasing their adhesiveness has been described.

It has been frequently observed that adherence of the leucocytes is not necessarily accompanied by emigration. The vascular response may subside and the leucocytes become detached from the vessel wall. In the simplest terms it looks as if the severity of the injury determines whether the leucocytes actually emigrate or not, but the term "severity of the injury" may again imply the release by the injured tissues of some special substances which induce the emigration by their direct action on the cells adherent to the walls. And this brings us to the question of chemotaxis.

Chemotaxis of Polymorphonuclear Leucocytes

The history of this subject is studded with more picturesque names than solid facts. The general pattern of thinking about chemotaxis seems to have been something like the following. If leucocytes accumulate at a certain site in the tissues, they must have been attracted chemotactically toward this site. Since leucocytes can be made to accumulate in the tissues without the intervention of bacterial infection the tissues must be able to release a substance chemotactic for leucocytes. This substance must be released when tissues are damaged and must constitute the general chemical principle underlying inflammation. Phlogosin (18), phlogistine (19), and leukotaxine (20) are perhaps the best known names in this connection.

The subject of chemotaxis of leucocytes has been reviewed in detail elsewhere (21). Therefore, the present discussion is limited to two aspects of the question: what types of technique can be regarded as reliable assays of chemotaxis in leucocytes and what substances have been shown to be chemotactic by acceptable techniques?

For reasons essentially similar to the ones discussed above, assays based on the injection of substances into the tissues and subsequent histological examination of the injection site must be rejected. In the first place such procedures again fail to distinguish between a direct action on the movement of the leucocytes and an indirect action by the liberation of some other substance from the tissues. Furthermore, a histological section can only reveal that leucocytes have accumulated in the tissues; it does not reveal how the accumulation occurred. An accumulation of leucocytes in the tissues might occur either in response to a chemotactic stimulus or because leucocytes which have emigrated from the vessels are immobilized or trapped or delayed at the site of accumulation. As it is precisely this distinction that one wishes to establish when one assays a substance for chemotactic effect, it is apparent that the injection procedure is unlikely to provide a clear answer.

The insertion into the tissues of capillary tubes filled with the test substance is also unacceptable. Apart from the fact that such tubes injure the surrounding tissues and might thus be responsible for the liberation of active substances from the injured cells, the accumulation of leucocytes within the tube is in itself no evidence of chemotaxis. This was pointed out by Pfoehl (22) as early as 1898 and was confirmed by Ruchlädew in 1910 (23). As a matter of fact, Leber (18), who first described chemotaxis of leucocytes, pointed out that some degree of leucocyte accumulation occurred in the capillary tube, whatever substance was put inside it: "at least I have never succeeded in finding a completely indifferent substance in this respect," as he himself puts it. Accumulation of leucocytes within the tube occurs as a result of convection currents set up at the mouth of the tube by physicochemical differences between the substance inside the tube and the substances outside it. Unless care is taken to eliminate such convection currents, which is very difficult *in vitro*, and apparently impracticable *in vivo*, the results obtained will have little significance.

The demonstration of a chemotactic effect involves the demonstration of a directional response on the part of the leucocytes to the substance tested. A chemotactic effect may be demonstrated *in vitro* if the leucocytes are alive and motile and the possibility of passive convection of the cells is eliminated. Various modifications of the slide-cover slip technique meet these requirements, but it should be emphasized that, just as in experiments *in vivo*, the accumulation of leucocytes about the test object or test substance is not in itself evidence of chemotaxis. It must be shown that the test substance has

actually produced a directional response in the leucocytes. This may be done by cinemicrography, as the unsurpassed films of Comandon (24) illustrate, or by recording the movement of the leucocytes by means of a camera lucida, as practiced by McCutcheon and his colleagues (25, 26), or, most conveniently and accurately, by the dark-ground trace technique. Figure 1 illustrates the random movement of leucocytes recorded as traces under dark-ground illumination. Figure 2 illustrates a chemotactic response to a clump of *Staphylococcus epidermidis* (*Staphylococcus albus*) recorded in the same way. It will be seen that the traces converge on the test object. There has been a clear-cut directional response on the part of the cells which have moved in virtually straight lines toward the bacterial colony.

The demonstration of chemotaxis *in vivo* must fulfill the same criteria as the demonstration *in vitro*: a directional response on the part of the cells must be shown. To our knowledge the only techniques which would permit such a demonstration are transparent chamber methods such as the rabbit ear chamber. It is possible in such chambers to observe whether the movement of the leucocytes from the vessels and in the tissues is random or whether the cells show a directional response; but transparent chamber techniques so far have the serious limitation that it is not possible to introduce a test substance into the chamber without trauma. Perhaps the hamster cheek pouch will prove to be more suitable.

It is generally agreed that a wide variety of bacteria are chemotactic for polymorphonuclear leucocytes. The old question of whether the bacteria themselves produce the chemotactic substances or whether they merely induce the liberation of such substances from the plasma or the tissue cells may, in one respect, be regarded as settled. There is no doubt that bacteria may produce a chemotactic response in leucocytes in the absence of any tissue cells or body fluids. It is to be noted that many varieties of microorganism produce a chemotactic response in the polymorphonuclear leucocyte, including organisms such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* that produce lesions in which monocytes predominate. It is not known whether each type of bacterium produces a different chemotactic substance or whether the same chemotactic substance is responsible for the effect in all types of bacteria. The best evidence today points to bacterial polysaccharides as the active substances, and the fact that starch grains are also chemotactic perhaps lends support to this view. But the evidence is not conclusive. This is one apparently straightforward problem which should be soluble with the techniques currently available. It is possible to assay substances for chemotactic effect with accuracy *in vitro*, and it

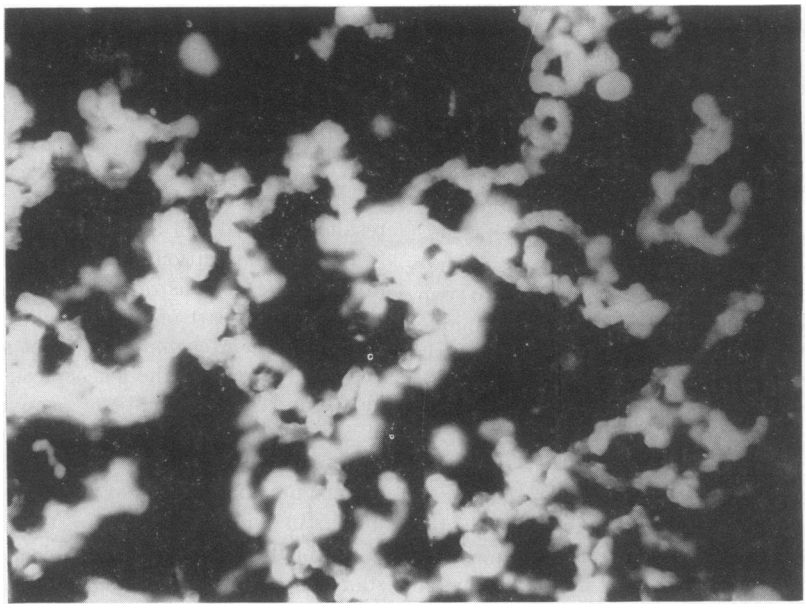

Figure 1. Migration pattern of polymorphonuclear leucocytes moving at random, as recorded by the dark-ground trace technique.

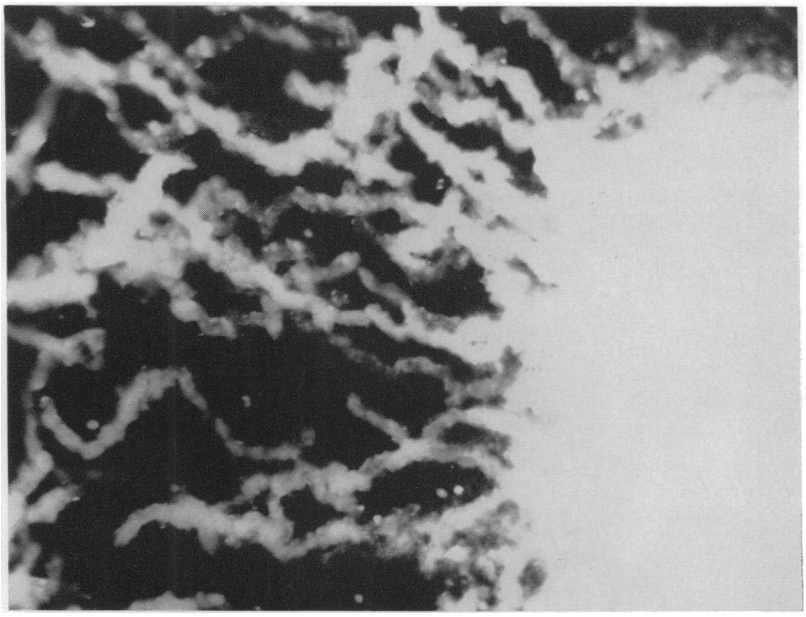

Figure 2. Effect of a clump of *Staphylococcus epidermidis* (*Staphylococcus albus*) on the migration pattern of polymorphonuclear leucocytes. The clump of bacteria is on the right; the leucocytes have moved directly toward it.

seems unlikely that separation and purification of the active substance or substances is beyond the powers of contemporary analytical methods.

Do injured tissues release substances which are chemotactic for leucocytes? If we admit as evidence only the data obtained by acceptable techniques, it can be said that to date no substance produced by or extracted from uninfected tissue has been shown to be chemotactic for leucocytes. Some years ago we tested a large number of tissue breakdown products for their chemotactic effect and failed to demonstrate chemotaxis for a single one provided the preparations remained free from bacterial contamination. In figure 3 the dark-ground pattern traced by the leucocytes in the presence of a piece of partially digested muscle is shown; it will be seen that the direction of movement of the leucocytes is uninfluenced by the presence of the muscle. This figure may be compared with figure 2 in which the effect of a clump of *Staphylococcus epidermidis* (*Staphylococcus albus*) is shown. However, one must be wary of the negative result. It is possible that the chemotactic substance elaborated by the tissues is extremely labile, or that the conditions *in vitro* do not simulate the conditions *in vivo* closely enough. However, these possibilities are made unlikely by the fact that under the best available conditions chemotaxis cannot be observed *in vivo* as a result of aseptic injury of tissues. It has been shown by Sanders (27), and in more detail by Allison *et al.* (28), that in the rabbit ear chamber the emigration of leucocytes produced by a localized thermal injury and the subsequent movement of the cells in the tissues show no evidence of being directed by a chemotactic influence. The cells appear to move in a completely random way, although after many hours they begin to accumulate at the site of the injury.

The present position may therefore be summarized in the following way. It has not been decided whether the initial vascular response in inflammation can be produced by the injurious stimulus itself or whether it is produced by the release of some special substance or substances from the injured tissues. Of the many substances which have been canvassed for this latter role none has the support of a compelling body of experimental evidence. The adherence of the cells to the endothelium may, but does not necessarily, involve a change in the adhesiveness of either the leucocytes or the endothelium, although if such a change does occur it is more likely to occur in the endothelium than in the leucocyte. The emigration of the leucocytes from the vessels is probably not owing to any chemical substance acting directly on the leucocytes; in any case there is no evidence that chemotaxis is involved in the emigration of

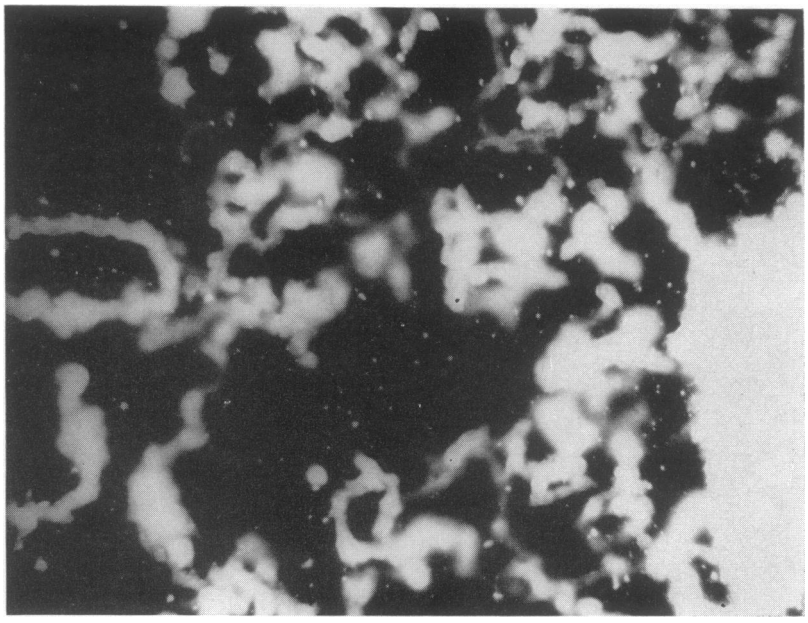

Figure 3. Random migration pattern of polymorphonuclear leucocytes in the presence of a fragment of muscle which had been subjected to the action of pepsin for 4 hr. The fragment is on the right.

the cells through the vessel walls. Once the cells are out in the tissues their movement may be influenced by chemotactic substances, but there is no acceptable evidence that chemotactic substances are produced by the tissues themselves. A wide range of microorganisms produce chemotactic substances, but the identification of these substances has not been achieved; there are some indications, but no rigid proof, that they are polysaccharide in type.

Emigration of Monocytes

Monocytes, or cells apparently derived from monocytes, gradually replace polymorphonuclear leucocytes in the later reparative stages of inflammation. Similar cells are the predominant phagocytes operative in the process of wound healing; and they are characteristically associated with certain types of bacterial infection such as leprosy and tuberculosis. If it is assumed that the mononuclear cells seen in the tissue lesions are at least in large part monocytes which have emigrated from the blood, there are three possible mechanisms that could account for the selective accumulation.

a. The conditions in the tissues provide a stimulus, chemical or otherwise, which induces a preferential adherence of monocytes rather than polymorphonuclear leucocytes to the endothelium of the inflamed capillaries, thus resulting in a preferential emigration of monocytes.

b. Monocytes and polymorphonuclear leucocytes emigrate together but some substance is present in the lesion which is selectively chemotactic for the monocytes, thus causing an accumulation of these cells rather than polymorphonuclear leucocytes.

c. Monocytes and polymorphonuclear leucocytes emigrate together and both accumulate in the lesion, but the conditions in the lesion are such that the polymorphonuclear leucocytes are gradually removed, whereas the monocytes remain.

Before considering these three possibilities a few remarks must be made about the assumption that most of the mononuclear cells seen in these lesions are derived from the blood and about the relationship between the numbers in the blood and the numbers in the lesions. Although it may not be true for lower animal forms, there seems to be a good case for believing that in mammals the accumulations of mononuclear cells seen in the tissues are owing to emigration of monocytes from the blood. The "tissue" macrophages, if they are not themselves originally derived from the blood, are in most situations too few in number to account for the large accumulations of mononuclear cells which can occur, unless the tissue

macrophages undergo a series of mitoses at the site of the lesion. But mitoses of mononuclear cells in such lesions are rarely seen. Where studies of the pathogenesis of mononuclear lesions have been made in living preparations, as, for example, in the investigations of Dodson *et al.* (29) on experimental tuberculosis in the rabbit ear chamber, the observations leave little doubt that the mononuclear cells originated from the blood stream. However, no consistent correlation can be found between the number of circulating monocytes and the number of mononuclear cells found in the lesions. Although in some conditions, for example, acute experimental tuberculosis or *L. monocytogenes* infection in animals, the presence of numerous mononuclear lesions in the tissues is associated with a monocytosis of varying degree, it is certainly possible to have mononuclear lesions of various sorts in the tissues without any accompanying monocytosis. Although it is possible that a monocytosis may have some bearing on the number of monocytes which emigrate from the vessels, monocytosis itself is neither a necessary condition for, nor a sufficient explanation of, the pathogenesis of the mononuclear lesion.

Selective Emigration of Monocytes

Although a mechanism of this sort would perhaps be the most simple and the most plausible one, there is unfortunately almost no evidence in support of it. Although there are a few descriptions in the literature of monocytes, among other cells, adhering to the walls of blood vessels, there are no observations of a selective emigration of monocytes from the vessels; and no stimulus has yet been devised which will provoke a selective emigration of this sort under experimental conditions. Almost all workers are agreed that microorganisms which eventually give rise to a mononuclear lesion produce in the first place a cellular response which is predominantly polymorphonuclear. It is, as a matter of fact, difficult to see how a vascular response which permitted monocytes to adhere to the vessel wall could avoid a concomitant adherence of polymorphonuclear cells. There has, however, been one recent observation which should serve as a caveat. Poole and Florey (30) in studies on the pathogenesis of aortic lesions in cholesterol-fed rabbits observed monocytes adherent to the endothelium of the aorta overlying the lesions and also migration of monocytes through the endothelium (figure 4). It is not clear whether the monocytes have collected in the subendothelial lesion and passed through the endothelium into the blood stream, or whether circulating monocytes have adhered to the endothelium and passed into the subendothelial tissues, but in either case the collection of

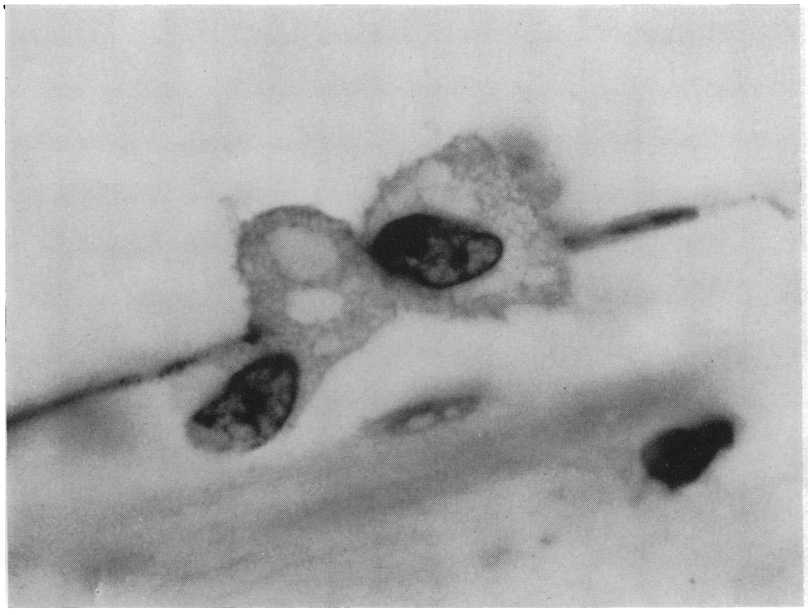

Figure 4. Macrophages passing through the endothelium of the aorta of a rabbit (30)

monocytes occurred without any observable polymorphonuclear accompaniment. Although conditions in the aorta may have little connection with the sequence of events in the capillaries, the observations of Poole and Florey mean that the possibility of selective emigration of monocytes cannot be entirely ruled out, despite the absence of evidence in support of such a mechanism.

Selective Chemotaxis of Monocytes

Until a few years ago it had not been conclusively demonstrated that the monocyte was subject to chemotaxis, and the literature abounded in statements to the effect that the monocyte showed a more feeble form of chemotaxis than the polymorphonuclear cell. By the use of the dark-ground trace technique it has now been unequivocally established that the monocyte is subject to chemotaxis. Figure 5 shows the traces made by monocytes moving at random and figure 6 shows the traces made by the cells in the presence of a clump of *M. tuberculosis*. It will be seen that the traces made by the cells converge directly on the clump of bacteria. By this technique it was shown that a large variety of microorganisms, including those normally associated with polymorphonuclear lesions, were chemotactic for the monocyte. As with the polymorphonuclear leucocyte, sterile tissue breakdown products were, however, found not to be chemotactic, as is illustrated in figure 7.

Over a very wide range of test objects it was found that the polymorphonuclear leucocyte and the monocyte reacted chemotactically to the same stimuli. No substance was found which was chemotactic for one cell without also being chemotactic for the other. Chemotaxis is thus a nonspecific response which both cells exhibit toward certain types of substances, and any explanation of mononuclear accumulation based on differential chemotaxis must at present be regarded as having no experimental foundation.

Replacement of Polymorphonuclear Leucocytes by Monocytes within the Lesion

On the whole, this type of mechanism seems now to be the most plausible, although plausibility should not be confused with fact and there is too little experimental evidence to permit one to decide what the facts are. If, as seems to be generally agreed, *Staphylococcus aureus* and *M. tuberculosis* produce initially the same predominantly polymorphonuclear response, and if differential chemotaxis plays no part in determining the subsequent evolution of the lesion, it seems probable that this evolution will be determined by the type of relationship established between the invading

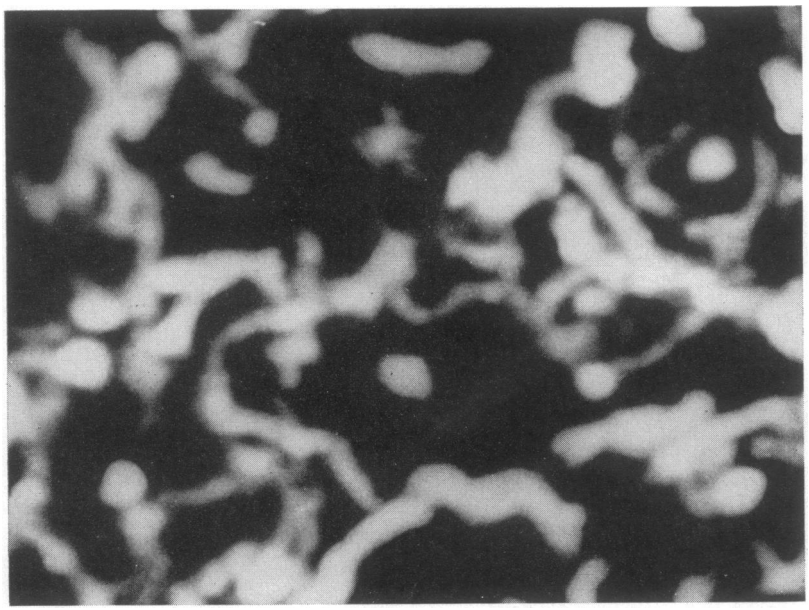

Figure 5. Migration pattern of monocytes moving at random

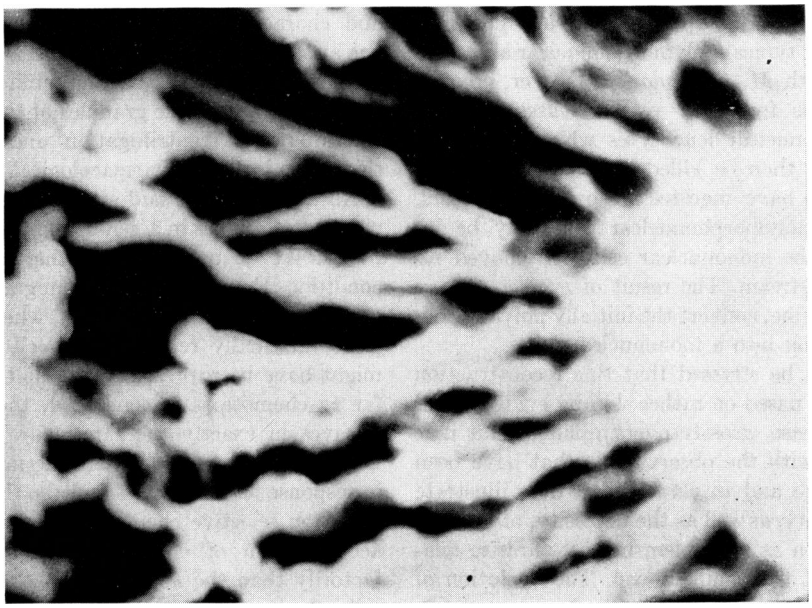

Figure 6. The pattern traced out by the monocytes in the presence of a clump of *Mycobacterium tuberculosis*; all the traces within a certain range of the clump converge directly upon it.

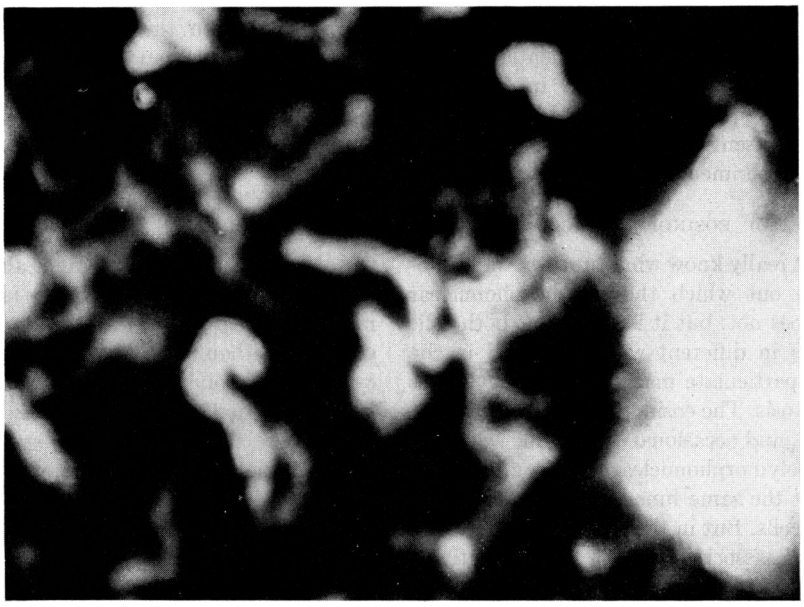

Figure 7. The pattern traced out by the monocytes in the presence of a fragment of autolyzed muscle; the cells in the vicinity of the fragment have moved in an entirely random way.

bacteria and the phagocytic cells. *S. aureus*, which multiplies rapidly, has a predominantly extracellular habitat, and produces an exotoxin, will continue to irritate the surrounding capillaries until the infection is brought under control. *M. tuberculosis*, which grows slowly, has a predominantly intracellular habitat, and produces no exotoxin, will cease to irritate the surrounding capillaries once the bacteria have been ingested. The result might therefore be that with *S. aureus*

emigration of polymorphonuclear leucocytes continues and a typical polymorphonuclear abscess is formed. With *M. tuberculosis*, however, emigration of cells from the vessels ceases and the polymorphonuclear leucocytes which have emigrated may then be killed by the mycobacteria which they have ingested or by other factors. The dead polymorphonuclear cells may be ingested by the mononuclear cells or removed *via* the lymph stream. The result of such a process may, with time, convert the initially polymorphonuclear lesion into a mononuclear one.

It should be stressed that this reconstruction of events is based on rather skimpy evidence and is, in any case, grossly oversimplified. But it is consistent with the observations that have been made *in vivo* and *in vitro*, and it does illustrate the possibility, as well as the necessity, of treating this question as a problem in host-parasite relationships at the cellular level. The evolution of the lesion must in any individual case be determined by the interplay of many factors of the sort that have been outlined, and it seems extremely improbable that any explanation involving only one factor will prove to be a sufficient explanation. One factor may be mentioned that has in the past been canvassed in this way, namely, that the pH of the lesion determines the type of cell present; this can now be dismissed for want of experimental support.

MECHANISM OF EOSINOPHILE ACCUMULATION

We do not really know what function the monocyte carries out which the polymorphonuclear leucocyte does not, but it is clear that both cells are, perhaps in different ways, involved in the removal of particulate material from the tissues by phagocytosis. The eosinophile leucocyte is also a phagocyte, and occasional eosinophile cells may be seen in polymorphonuclear lesions, apparently carrying out the same functions as the polymorphonuclear cells. But in the characteristic eosinophile lesions associated with hypersensitivity reactions, large numbers of eosinophiles accumulate in the tissues and do not there seem to be engaged in any phagocytic role. We do not have any idea at all what the eosinophile is doing in these accumulations. In a sense our ignorance about the function of the eosinophile is even more surprising than our ignorance about the lymphocyte; for, whereas the small lymphocyte has very little cytoplasm and no unusual cytoplasmic structures, the eosinophile has exceedingly large and characteristic granules, whose composition has apparently never seriously been investigated. It should not be an exceedingly difficult technical problem to obtain the granules of the eosinophile by differential centrifugation and to examine their chemical and pharmacological properties.

All that has been said about the emigration of monocytes applies in a general way to the eosinophiles. We do not know whether under certain conditions there is a selective emigration of eosinophiles from the vessels, or whether eosinophiles gradually replace other cell types which might have been present in the initial lesion. As far as chemotaxis is concerned, the eosinophile behaves in exactly the same way as the polymorphonuclear leucocyte and the monocyte, and in response to the same stimuli; so that no theory based on selective chemotaxis could explain the accumulation of eosinophiles any more satisfactorily than the accumulation of mononuclear cells. As in the case of mononuclear lesions, accumulations of eosinophiles in the tissues are often associated with an eosinophilia in the blood, but the degree of eosinophilia is not in itself sufficient to explain the predominance of eosinophiles in the tissues, and in many cases accumulations of eosinophiles are present in the tissues without any increase in the number of circulating eosinophiles.

MECHANISM OF LYMPHOCYTE ACCUMULATION

It will serve no purpose to present the traditional recitation of our ignorance about the function of the lymphocyte. We do not know any more about the mechanism of lymphocyte accumulation than about the mechanisms of monocyte or eosinophile accumulation, and the same kind of alternatives suggest themselves. But the lymphocyte does pose certain special problems which are not posed by the other cells. The polymorphonuclear leucocyte, the monocyte, and the eosinophile all have certain surface characteristics in common: they all adhere readily to wettable surfaces, they are all phagocytic cells, and they all exhibit chemotaxis in response to the same sorts of stimuli. If a vascular reaction is produced that results in the adherence of polymorphonuclear leucocytes to the vessel walls, it would seem probable that this type of reaction would also result in the adherence of monocytes and eosinophiles. In the rabbit ear chamber the adherence of some monocytes is in fact often seen when an inflammatory response is produced. But

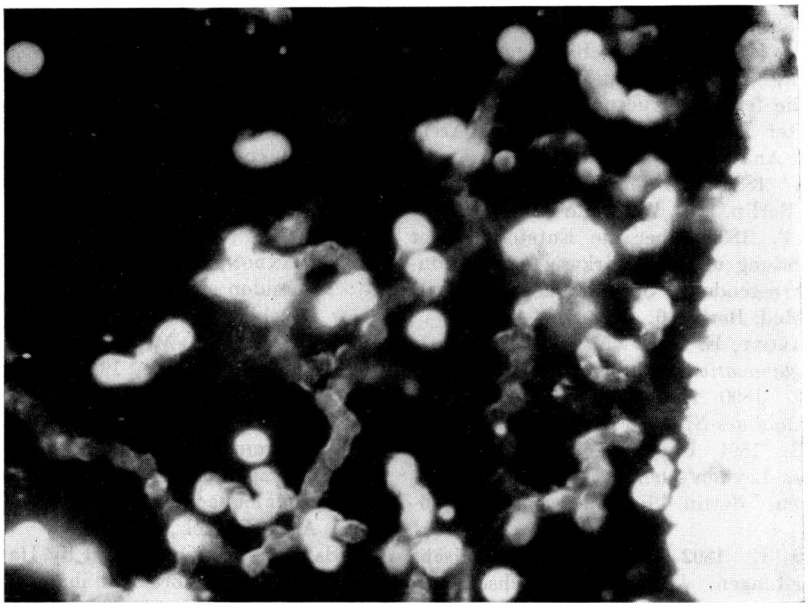

Figure 8. Pattern traced out by lymphocytes in the presence of a clump of *Mycobacterium tuberculosis*. The clump is on the right. Traces show no tendency to converge on the bacteria. The round dots represent stationary cells.

the surface characters of the lymphocyte are quite different. This cell does not adhere to wettable surfaces, it is not phagocytic, and it does not exhibit chemotaxis in response to any stimuli yet tried. Figure 8 shows the random movement of lymphocytes in the presence of *M. tuberculosis*, which produces a clear-cut chemotactic response in the other cell types. It might be supposed, therefore, that the vascular reaction which produces adherence of the other leucocytes might not produce adherence of lymphocytes. This supposition is, on the whole, borne out by observation. Although lymphocytes have occasionally been seen to adhere to vessel walls in the ear chamber, it is fairly clear that the conditions which provoke the adherence and emigration of polymorphonuclear cells do not provoke the adherence of lymphocytes. This is best illustrated in the rat, where, despite the fact that lymphocytes are the most numerous of the circulating leucocytes, acute inflammation results in a predominantly polymorphonuclear emigration, just as it does in animals in which the polymorphonuclear cell is the most numerous of the circulating leucocytes.

In thinking about possible mechanisms of lymphocyte accumulation there seems to be no alternative to some form of selective emigration. The increasingly convincing body of experimental evidence implicating cells of the lymphoid series in antibody formation and the association of lymphocytes with certain types of antigen-antibody reaction in the tissues leads one to speculate that some form of combination between antigen and antibody might be involved in the adherence of lymphocytes to vessel walls and hence in their emigration into the tissues. But this is just one of many possible guesses. A clearer understanding of how lymphocytes accumulate in the tissues will perhaps emerge when we have a clearer understanding of what they are doing there.

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DISCUSSION

The production of a chemotactic substance by a wide variety of bacteria, which attracts leucocytes capable of causing their destruction, seems to be an extraordinary biological phenomenon (MacLeod, Philadelphia). On the other hand, it would be very clever of leucocytes to evolve a mechanism for recognizing the substances that are of interest to them. This reaction can be traced back to the most primitive feeding habits of cells, as described in the classical studies of Metchinkoff (*La Pathologie Comparée de l'Inflammation,* E. Metchnikoff, Masson et Cie. (Paris, 1892), and it would probably not be difficult to establish a link between this and the specialized cells with phagocytic activity in complex animal species (Harris, England).

There is no evidence that the attraction of

phagocytic cells to bacteria or other foreign material is attributable to a failure of repulsion associated with differing electrical charge or lack of repellent substance. For example, electrophysical studies have failed to demonstrate repulsion of phagocytic cells and no natural substance has been described that repels leucocytes and which, if interfered with, can account for chemotaxis. Nevertheless, these possibilities have not been absolutely excluded (Rowley, England).

In several infections, such as those caused by pneumococcus and meningococcus, inflammation characterized by leucocytic infiltration may not be observed early in the process of bacterial invasion and multiplication but appears after a period of time. In addition, it is known that infection by the typhoid bacillus may not induce a leucocytic response except when the infection is in certain areas, as in the peritoneal cavity or bone. These histopathological observations might at first glance suggest that substances produced as a result of interaction between the host's tissues and the microorganism are chemotactic rather than substances elaborated by the microorganism itself. However, it is important to remember that not all bacteria have been demonstrated to have chemotactic properties and, furthermore, tissue injury resulting from infection certainly may, in and of itself, induce a leucocytic reaction. In short, the chemotaxis induced by bacteria undoubtedly is not the only mechanism of activating phagocytic cells. The factors responsible for the infiltration of tissue by polymorphonuclear leucocytes are most likely complex, and the chemotactic activity of bacteria will not always or even usually explain this manifestation of inflammation (Goodpasture, Nashville).

It is possible that species differences may exist in the response of leucocytes to the chemotactic effect of bacteria and there may even be differences between the phagocytes from different members of the same species. However, the studies described have been done largely with polymorphonuclear leucocytes from the blood of man, rabbits, and guinea pigs; monocytes from rabbits, guinea pigs, and rats; eosinophiles from man and guinea pigs; and lymphocytes from rats and rabbits. Little effort has thus far been made to evaluate genetic influences upon chemotaxis (Gowen, Iowa).